UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

010739

MEMORANDUM

JAN - 6 1994

SUBJECT: Difethialone, New Chemical
Anticoagulant Rodenticide

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

From: Ray Landolt 12/30/93
Review Section I
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Health Effects Division (7509C)

Barcode D193280
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TO: Robert Forrest, PM 14
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THRU: Mike Ioannou, Section Head
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J M Ioannou 12/30/93

M van Gemert 1/5/94

Registrant: LiphaTech, letters of January 8, 1993, February 26, 1993 and September 14, 1993.

Action Requested: 1. The registrant has submitted additional information to upgrade previously reviewed studies classified Supplementary in DER 006460 and 007523.

2. Review the following studies on the technical material (98.4%) in support of registration (EPA No. 7173-ENU).

(81-4) Primary Eye Irritation, Study No. 92N1079
(81-6) Dermal Sensitization, Study No. 008-0010R
(83-3) Developmental - Rat, Study No's 509202 and 504211
(83-3) Developmental - Rabbit (Test Protocol)
(84-2a) Gene Mutation, Study No. 811471
(84-2b) Chromosomal Aberr., Study No's 903325 and 15136-0-449
(84-2b) Micronucleus In Vivo - Mice, Study No. 15136-0-455
(86-1) Domestic Animal Safety Antidotal Treatment-Dogs
Study No. 2624-100

3. Review the following studies conducted with a pelleted bait formulation (0.0025%) in support of registration of Pellets (EPA No. 7171-ENL) and Pellets Place Packs (EPA No. 7173-ENA).

(81-4) Primary Eye Irritation, Study No. 92N1080
(86-1) Domestic Animal Safety Antidotal Treatment-Rats
Study No. 2624-101.

Recommendations: The data base in support of registration of the technical and pelleted formulations are incomplete.

1. Data waiver was granted for a General Metabolism Study with Toxicology Review (Tim McMahon) of June 2, 1992. However, a modified metabolism study is required to characterize the 1/2 life of difethialone (Tox Review of February 5, 1990).

2. The test protocol for (83-3) Developmental Toxicity Study in the rabbit is acceptable in Tox. Review (Steve Dapson) of December 22, 1993.

3. The registrant has concluded that an Acute Inhalation Toxicity Study (81-3) with the 0.0025% formulation is not necessary in Tox. Review (Tim McMahon) of June 2, 1992. With particular concern for respirable fines in the package, the rational for this conclusion is requested.

Conclusions: The following studies are acceptable and support the registration of difethialone.

Technical, 98.8%

Study	MRID	DER	Classification	Toxicity Category
(81-1) Acute Oral-Rat LD_{50} 0.55 mg/kg	402689-03 426877-04	006460 This Review	Supplementary to Guideline	1
Acute Oral- Mice LD_{50} 1.29 mg/kg	402689-04 426877-05	006460 This Review	Supplementary to Guideline	1
(81-2) Acute Dermal-Rat LD_{50} 6.5 mg/kg	402689-06 426877-06	006460 This Review	Supplementary to Guideline	1
(81-3) Acute Inhalation-Rats LC_{50} <0.011 mg/L	402689-07 426877-07	006460 010018	Supplementary to Guideline	1
(81-4) Eye Irritation-Rabbit Slight irritant	402689-08 426877-08	006460 This Review	Supplementary to Guideline	3
(81-4) Eye Irritation-Rabbit Slight irritant	402689-09 426877-09	006460 This Review	Supplementary to Guideline	3
(81-4) Eye Irritaion-Rabbit Moderate irritant	426281-06	This Review	Guideline	2
(81-6) Dermal Sensitization	426877-01	This Review	Guideline	Negative

Study	MRID	DER	Classification
(82-1) 90-Day Oral-Rat	407914-02 426877-10	007523 This Review	Supplementary to Minimum

Three groups of 20 rats/sex/group were dosed orally by gavage 7-days/week for 13 weeks at 0, 0.002, 0.004 or 0.008 mg/kg/day. Ten rats/sex/group were sacrificed during week six for determination of hematology and clinical chemistry parameters.

NOEL-0.004 mg/kg/day

LOEL- 0.008 mg/kg/day with mortality of 3/10 males during the last two weeks of the study. Hematuria, epistaxis, decreased activity and pale in appearance were reported for these animals during days 90 to 93. Coagulation indices were significantly ($p<0.01$) prolonged by 2 to 3 times in females and 13 to 16 times in males as compared to the control values.

(83-3)	Deveolpmental-Rat	422038-02 433038-01	This Review	Minimum

Four groups of 25 pregnant female rats/group were dosed orally by gavage at 0, 12.5, 25 or 50 mg/kg/day on days 6 through 17, inclusive.

Maternal Toxicity NOEL $\geq$ 50 ug/kg/day

LOEL > 50 ug/kg/day

Developmental Toxicity NOEL $\geq$ 50 ug/kg/day

LOEL > 50 ug/kg/day

Study	MRID	DER	Classification
(84-2a) Gene Mutation-HGPRT locus CHO cells	420650-08	This Review	Acceptable

Not mutagenic at 5.1, 10, 51, 100 or 210 ug/ml with and without S9 activation. Severely cytoxic (<10% survival) at $\geq$ 150 ug/ml.

(84-2b)	Chromosome Aberration Human Lymphocytes	420650-07	This Review	Acceptable

Not Mutagenic at 51, 100 or 210 ug/ml with or without S9 activation. Mitotic supression at 210 ug/ml with or without S9 activation.

Study	MRID	DER	Classification
(84-2b) Chromosome Aberration Human Lymphocytes	426281-07	This Review	Acceptable

Not mutagenic with a 20 hr. delayed harvest to 5 ug/ml without activation or to 449 ug/ml with S9 activation. Negative with a 30 hr. delayed harvest to 10.1 ug/ml without S9 activation. Severe cytoxicity at 299 ug/ml with activation or at 10.1 ug/ml without S9 activation.

(84-2b) Micronucleus In Vivo Mice	426281-08	This Review	Acceptable

In Vivo micronucleus assay in mice dosed for 3 consecutive days IP at 20 mg/kg was negative for micronucleus induction of bone marrow cells. MTD was demonstrated with mortality at >1.0 mg/kg.

(86-1) Domestic Animal Safety Antidotal Treatment-Dogs	421143-01	This Review	Acceptable

Difethialone (98.5%), administered orally by capsule to 5 beagle dogs/sex at 40 mg/kg, prolonged prothrombin time by 3 times the pretreatment value by Day 3 (48 hours) of the study. The initial dose of vitamin K_1 was administered intravenously at 5 mg/kg on Day 3. A second dose of vitamin K_1 was administered orally on Day 3 at 5 mg/kg followed by twice daily oral doses of 5 mg/kg through Day 9, then once daily on Day 10 through Day 30. This regimen was effective in reversing the anticoagulant effects to the pretreatment values following the final dose of vitamin K_1 on Day 30 through termination of the study on day 50. All animals survived to the termination of the study.

Pelleted Bait 0.0026%

(81-4) Eye Irritation-Rabbit	426280-01	This Review	Guideline
(86-1) Domestic Animal Safety Antidotal Treatment-Rats	420650-13	This Review	Acceptable

Three groups of 10 male rats/group were fed difethialone (0.0026%) pelleted bait for 24, 48, or 72 hours, respectively. This represents a group mean daily intake of approximately 2.0 mg/kg/day of difethialone. Vitamin K_1 was an effective treatment when administered immediately following the 24 hour exposure, but marginally effective in reversing the hemorrhagic signs of toxicity when administered following the 48 of 72 hour exposure.

Consideration Given This Request:

(82-1) Subchronic Oral Toxicity - Rat
Study No. 85.01.LM2219Rpp, December 11, 1986, MRID 407914-02

A 90-Day rat oral toxicity study submitted by LiphaTech June 1988 was reviewed (DER 007523) October 2, 1989 and Classified Supplementary with a request for additional information so this study may be upgraded. This study was conducted in two parts. The main study consisted of four groups of 20 rats/sex/dose of difethialone administered by gavage at 0, 2, 4, or 8 ug/kg/day. A satellite study was conducted with four groups of 10 rats/sex/dose of difethialone administered by gavage at 0, 16, 32, 64 or 128 ug/kg to determine the dose to time of death relationship. Hematology, clinical chemistry and urinalysis were not determined in the satellite study.

The following deficiencies were cited in DER 007523 accompanied by the registrant's response (MRID 426877-10).

1. Deficiency: Gross observations of the control animals were not reported. These are necessary for comparison to those observations reported for the test groups ie, female rats of the low-, mid-, and high-test groups were perturbed (excited) on Day 16. Was this observation consistent with the control females?

   Response: Tables submitted for the gross observations of the main study show that the excitation reported in the test animals was not evident in the main study controls, but was observed in the female satellite controls during Day 14 of the study.

   Reviewers Response: The data provided are acceptable. This deficiency is resolved.

2. Deficiency: The references for the absolute and coagulation indices were requested.

   Response: The method, units and abbrevations used for laboratory examinations are described in Appendix 4 of this addendum (MRID 426877-10).

   Reviewers Response: The data provided are acceptable. This deficiency is resolved.

3. Deficiency: Triglyceride and magnesium parameters were not measured at the termination of the study. The rational for omitting the triglyceride and magnesium determinations at the termination of the study were requested.

   Triglyceride and magnesium values were significantly decreased during week 6 at the high dose.

   Response: With the death of 3 animals and survivors in poor health at the high dose, difficulties in withdrawing sufficient blood for these determinations were cited. The study author "decided to limit the clinical investigations at week 13 to those recommended by the Pesticide Assessment Guidelines, Subdivision F. Hazard Evaluation; Human and Domestic Animals."

   Reviewers Response: The information provided is acceptable. This deficiency is resolved.

4. Deficiency: The rational for omitting urinalysis at the termination of the study was requested.

   Response: The study author cited the testing guidelines for "Urinalysis is not required on routine basis but only when there is an indication based on expected or observed toxicity" and "decided to limit the clinical investigations at week 13 to those recommended by the Pesticide Assessment, Subdivision F. Hazard Evaluations, Human and Domestic Animals".

   Reviewers Response: During week 6 at the high dose, urinary volume increased three fold accompanied by an elevated pH was reported as compared to the control values. As cited previously this parameter is required "when indicated by expected or observed activity". Such activity was demonstrated during the 6-week interval. This deficiency is not resolved and does not permit a "Guideline" classification of the data.

4. Deficiency: In this 90-day Oral Toxicity Study, reference is made of a Pharmacokinetic Study in which plasma and liver tissue was taken from 10 rats/sex/dose during week 6 for determination of difethialone levels. The methods and results of this study were requested with DER 007523.

Response: The registrant has identified this study entitled "Toxi-Kinetics and Metabolism:1, Study Performed with the Molecule Marked 14C. 2, Study Performed with the Cold Molecule" submitted 10-18-91, MRID 420650-10.

This pharmacokinetic study was subject to New Chemical Screening Review (Tim McMahon) of March 17, 1992 with the conculsion that it did not meet acceptance criteria due to too few animals tested. The methods for the conduct of this study were not provided with this response. However, the tissue levels in the liver of rats fed difethialone by gavage for 92 days are summarized in the following table from Appendix 6, p. 49, of this response (MRID 426877-10).

Dose Levels of Difethialone Administered Orally (mg/kg/day)

Liver Group Mean Concentration in mg/kg	0.002	0.004	0.008
Males	0.62	0.91	1.0
Females	0.76	1.31	1.52

Reviewers Response: The information provided satisifes the data request of October 2, 1989 (DER 007523). With this information and that of Tox. Review of March 17, 1992 resolved this deficiency.

5. Deficiency: A summary of the macroscopic observations (in tabular form) of the control, low- and mid-test groups were requested for the 90-day study.

Response: The registrant has submitted gross necropsy findings in tabular form for the terminal observations of the control, low-, mid- and high-test groups.

Reviewers Response: No dose related macroscopic observations were reported for the low and mid dose levels. The data provided are acceptable. This deficiency is resolved.

6. Deficiency: The signs of toxicity for female rats at the 0.032 mg/kg (in the satellite study) were not reported and should be submitted.

   Response: The signs of toxicity were reported in Table 3 of the final report (page 64) and were quite similar to those recorded for male rats at this level.

   Reviewers Response: The data provided are acceptable. This deficiency is resolved.

7. Deficiency: Gross observations of the control animals (in the satellite study) were not reported and should be submitted.

   Response: The registrant has submitted the gross observations (behavior and appearance) for the 90-day and satellite control animals.

   Reviewers Response: The data provided are acceptable. The deficiency is resolved.

Conclusion: The registrant has provided the additional information requested in Toxicology review (DER 007523) of October 2, 1989.

Classification of Data: From Supplementary to Minimum

Deficiency: Urinalysis was determined at the 6-week interval but not at the termination of the study.

This study satisfies the guideline data requirement (82-1) for a 90-Day Oral Toxicity Study in Rats.

NOEL - 0.004 mg/kg

LOEL - 0.008 mg/kg with mortality of 3/10 males, hematuria, epistaxis, decreased activity and pale appearance were reported in males at this dose level.

Coagulation indices were significantly ($p<0.01$) prolonged by 2 to 3 times in females and 13 to 16 times in males as compared to the control values.

Toxicology Profile for Technical Difethialone

Difethialone is anticoagulant rodenticide with proposed terrestrial and residential nonfood uses. The following data requirements for a "New Chemical" are consistent with FIFRA 88 data requirements for reregistration of anticoagulant rodenticides. The remaining 158.340 Toxicity Data Requirements (ie, chronic) have either been waived or are not required for the registeration of these rodenticides.

Toxicology Data Requirements for Technical Difethialone

Acute Testing:	MRID No.	DER	Satisified
81-1 Acute Oral Toxicity	402689-03 426877-04	006460 This Review	Yes
81-2 Acute Dermal Toxicity	402689-06 426877-06	006460 This Review	Yes
81-3 Acute Inhalation Toxicity	402689-07 426877-07	006460 010018	Yes
81-4 Primary Eye Irritation	426281-06	This Review	Yes
81-5 Primary Dermal Irritation	402689-09	006460 This review	Yes
81-6 Dermal Sensitization	426877-01	This Review	Yes
Subchronic Testing:			
82-1 90-Day Oral Toxicity	407914-02 426877-10	007523 This Review	Yes
Chronic Testing:			
83-3 Developmental-Rat	433038-01 422038-02	This Review	Yes
Mutagenicity Testing:			
84-2a Gene Mutation	420650-08	This Review	Yes
84-2b Chromosomal Aberration	4260650-07	This Review	Yes
84-4 Other Genotoxic Effects			No*
Special Testing:			
85-1 Metabolism			No**
86-1 Antidotal Treatment-Rat	420650-13	This Review	Yes
Antidotal Treatment-Dog	421143-01	This Review	Yes

* Waived for difethialone and other anticoagulant rodenticides
** A metabolism study is required to characterize the 1/2 life of difethialone. This is consistent for other anticoagulant rodenticides.

Toxicology Data Requirements for the pelleted bait (0.0025%) formulation

For nonfood use in tamper-resistant bait stations placed inside of homes, agricultural, commercial, industrial, public buildings and transport vehicles, (ships,trains,aircraft) and related port or terminal buildings.

Acute Testing:	MRID No.	DER	Satisified
81-1 Acute Oral Toxicity	402689-05	006460	Yes
81-2 Acute Dermal Toxicity	402689-06	006460	Yes
81-3 Acute Inhalation Toxicity			No*
81-4 Primary Eye Irritation	426280-01	This Review	Yes
81-5 Primary Dermal Irritation	402689-10	006460	Yes
81-6 Dermal Sensitization	402689-11	006460	Yes

* "The registrant indicated that an acute inhalation study would be performed for the 0.5% dry concentrate, but that such a study was not necessary for the 0.0025% pelleted formulation." Toxicology New Chemical Review (Tim McMahon) of June 2, 1992.